apoptosis via phosphrylation of p38. Stress-induced chondrocyte apoptosis was decreased due to inhibition of p38 MAP kinase activation when chondrocytes were incubated with SB203580 or p38 specific siRNA transfection. While, the phosphorylation of p38 MAP kinase was increased in OA chondrocytes. From the point of our results, down regulation p38 MAP kinase activation inhibited chondrocyte death induced by heat stress. Therefore, evaluation of the interaction between phosphorylation of p38 and chondrocyte apoptosis could be one of the keys to elucidating OA etiology.

Reference

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CROSS-TALK OF SUBCHONDRAL BONE OSTEOBLASTS AND ARTICULAR CARTILAGE CHONDROCYTES: A NEW INSIGHT IN UNDERSTANDING OSTEOARTHRITIS PATHOGENESIS

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Purpose: The purpose of this study was to address the question of how subchondral bone osteoblasts (SBOs) and articular cartilage chondrocytes (ACCs) interact with each other with respect to regulation of respective cells' phenotypic properties and in particular the involvement of mitogen activated protein kinase (MAPK) signalling pathways under normal and OA joint condition. We also endeavoured to test the influence of cross-talk between SBOs and ACCs isolated from normal and OA joint on matrix metalloproteinase (MMP) expression.
Methods: Tissues from the knees of OA patients and normal controls were collected to isolate SBOs and ACCs. The cellular cross-talk of SBOs and ACCs were studied by means of both direct and indirect co-culture systems, which made it possible to identify the role of both membrane bound and soluble factors. Histology, immunohistochemistry, qRT-PCR, zymography, ELISA and western blotting were some of the techniques applied to distinguish the changes in the co-cultured *vs.* non co-cultured cells. The MAPK signalling pathways were probed by using targeted MAPK inhibitors, and their activity monitored by western blot analysis using phospho MAPK specific antibodies.
Results: Our co-culture studies demonstrated that OA ACCs enhanced the SBOs differentiation compared to normal ACCs. We demonstrated that OA ACCs induced these phenotypic changes in the SBOs *via* activating an ERK1/2 signalling pathway. The findings from this study thus provided clear evidence that OA ACCs play an integral role in altering the SBO phenotype.
In the second study, we tested the influence of normal SBOs and OA SBOs on ACCs phenotype changes. The results showed that OA SBOs increased the hypertrophic gene expression in co-cultured ACCs compared to normal SBOs, a phenotype which is considered as pathological to the health and integrity of articular cartilage. It was observed that these phenotype changes occurred via de-activation of p38 and activation of ERK1/2 signaling pathways. These findings suggest that the pathological interaction of OA SBOs with ACCs is mediated by cross-talking between ERK1/2 and p38 pathways, resulting in ACCs undergoing hypertrophic differentiation.
Subsequent experiments to determine the effect on MMP regulation, of SBOs and ACCs cross-talk, revealed that co-culturing OA SBOs with ACCs significantly enhanced the proteolytic activity and expression of MMP-2 and MMP-9. In turn, co-culture of OA ACCs with SBOs led to abundant MMP-2 expression in SBOs. Furthermore, we showed that the addition of ERK1/2 and JNK inhibitors reversed the elevated MMP-2 and MMP-9 production which otherwise resulted from the interactions of OA SBOs-ACCs. Thus, this study has demonstrated that the altered interactions between OA SBOs-ACCs are capable of triggering the pathological pathways leading to degenerative changes seen in the osteoarthritic joint.
Conclusions: Our results has given clear *in vitro* evidence that the altered bi-directional communication of SBOs and ACCs may play a role in OA development and that this process was mediated by MAPK signalling pathways. Targeting these altered interactions by the use of MAPK inhibitors may provide the scientific rationale for the development of novel therapeutic strategies in the treatment and management of OA.

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CROSS-TALK BETWEEN TGFβ AND IL-1β SIGNALLING PATHWAYS: INVOLVEMENT IN OSTEOARTHRITIS

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Purpose: Interleukin-1β (IL1β) activity or its signaling cascade has been implicated in the pathogenesis of a number of diseases including rheumatoid arthritis or osteoarthritis. Dysregulated Transforming Growth Factor β (TGFβ) signalling is also involved in the osteoarthritis process. These two cytokines play antagonistic roles in cartilage homeostasis and several reports have highlighted cross-talk between these two pathways. Here, we investigated whether IL1β interferes with TGFβ signalling in human chondrocytes.
Methods: TGFβ1-induced gene expression was analyzed through plasminogen activator inhibitor 1 and p3TP-Lux induction in human articular chondrocytes. Receptor-activated Smad (RSmad) phosphorylation, TGFβ receptors, and Smad expression were determined by Western blotting and real-time reverse transcription-polymerase chain reaction techniques. Signalling pathways were investigated using specific inhibitors, messenger RNA (mRNA) silencing, and expression vectors.
Results: IL-1β impaired TGFβ signalling through TGFβ receptor type II (TβRII) down-regulation and Smad7 upregulation. IL-1β led to inhibition of TGFβ1-induced gene expression and Smad2/3 phosphorylation. Interestingly, TβRII overexpression restored the TGFβ response of human articular chondrocytes. However, this effect was transient, suggesting that a secondary mechanism was responsible for the alteration of the TGFβ response with long-term exposure to IL-1β.
IL-1β down-regulated TβRII expression at both protein and mRNA levels. It reduced TβRII transcription by inducing Sp3 via NFκB and its binding on core promoter at the expense of Sp1. In addition, it increased TRII protein degradation through receptor-mediated endocytosis by lipid raft and proteasomal pathway.
Furthermore, IL-1 strongly stimulated the expression of inhibitory Smad7. This effect was late (after 12h of incubation with IL1). It did not require de novo synthesis, and is dependent on NFkB pathway.
Conclusions: These findings clarify the regulatory process of IL-1β on TGFβ pathway. The IL1β-induced-impairment of TGFβ signaling could explain the reduced responsiveness of chondrocytes to TGFβ and therefore the cartilage breakdown during OA. Understanding this process provides new insights into the molecular mechanisms of osteoarthritis and may facilitate the identification of novel approaches for its treatment.

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EXPRESSION AND FUNCTION OF THE ARYL HYDROCARBON RECEPTOR IN GROWTH PLATE CARTILAGE CELLS IN VITRO

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Purpose: Growth and development of the musculoskeletal system is a complex process requiring intricate controls of cellular activity. Persistant environmental pollutants including ligands of the Arylhydrocarbon Receptor (AhR) such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or Benzo(a)pyrene (BaP, tobacco tar fraction) have been shown to elicit severe developmental defects in rodent cartilage and bone as well as growth retardation in humans. However, the status of AhR in cartilage is currently unknown. We investigated AhR expression and function in growth plate (GP) chondrocytes and assessed AhR ligands impact on cartilage processes.
Methods: GP chondrocytes were established in primary culture. Cells were challenged with Interleukin-1beta (IL-1B) for 6 or 20 hr at concentrations ranging from 0.05 to 2 ng/ml, then for another 20 hr with TCDD (5 nM) or BaP at 1 μM. IL-1B and AhR target genes were analyzed by qPCR and western blot. AhR localization was analyzed by immunocytochemistry
Results: GP chondrocytes expressed minor levels of AhR. AhR translocation by TCDD into the nucleus required normoxia and the presence of IL-1B. IL-1B induced AhR expression at the mRNA and protein levels and this induction was higher (2-fold) in normoxia than hypoxia. The IL-1B effect on AhR is transcriptional, through the AP-1 pathway.
Upon addition, IL-1B elicited a transient decrease (1h-6h) in Cyp1A1 mRNA